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CYCLICAL CHANGE IN PHASE OF ACTIVITY OF THE SUPPRESSOR-MUTATOR CONTROLLING ELEMENT IN MAIZE.

The nature and mode of action of controlling elements in maize has been discussed in a number of publications by the author and by others. Two or more elements may operate as a unit in the control of action of a specific gene, and each such set of interrelated controlling elements forms a system. Each system, in turn, operates quite independently of all others. Systems of controlling elements in maize were originally discovered because the individual members within each transposed from one location to another in the chromosome complement without losing their identifying characteristics in the process. By this means, it was possible to distinguish different systems of controlling elements and the manner by which each system operates in the control of gene action. Likewise, because of transposition of ~~the~~ component elements of a system, it ~~was likewise~~ is possible to examine the operation of a particular system at a number of different gene loci and conversely, to examine the operation of different systems at the same gene locus. It should be emphasized, however, that although transposition of controlling elements in maize made it possible, originally, to recognize their presence and their modes of operation, such transposition need not characterize the behavior of all controlling elements, for it is known

th t a controlling element that had previously undergone transposition may become fixed in location in the chromosome complement.

Recent discovery of systems of "controlling elements" in bacteria (Jacob, et al., 1960 a b) whose modes of action resemble those of some systems already examined in maize, eases the task of discussing controlling elements in maize. The systems described by Jacob are composed of two elements each. One of them, called the "operator", is located adjacent to the "structural gene". The latter, when activated, is responsible for the amino acid sequence of a specific protein whereas the former serves to control the activation of the adjacent "structural gene". The second element of the system, termed the "regulator", may be located close to the "structural gene" or it may be located elsewhere in the bacterial chromosome. It is responsible for the production of a repressor substance, -- not a protein --, that appears in the cytoplasm. The "operator" element, responds to changes in degree of effective action of the repressor substance by controlling the degree of activity of the structural gene in accordance with such changes. Each system is highly specific, for each operates quite independently of all others.

The resemblance of the "operator-regulator" systems in bacteria to

the systems of controlling elements in maize appears to be more than coincidental. The "Operator" in bacteria may be homologized with the controlling element in a system in maize that is located adjacent to the "structural gene". The "~~repressor~~ regulator" element in bacteria may be homologized with the element of a system in maize that is independently located in the chromosome complement. As in the bacteria, the element at the locus of the structural gene responds to ~~it~~ alters activity of the "regulator" element by inducing modification of ~~the~~ action of the structural gene. In maize, the response of the "operator" element to change in the effective action of the "~~repressor~~ regulator" element may result in controlled types of mutation at the locus of the "structural gene", or, in some cases, it may respond merely by "turning on" or "turning off" the action of the structural gene. That these two apparently quite different types of effects ~~pr~~oduced by the "operator" in response to the "regulator" are merely two aspects of the type of control of gene action by a single "operatore" will be made evident in this report. It is probable, therefore, that the basic mechanism of action of controlling elements is alike in all organisms, even though the responses of the structural gene to such controls may appear to be quite diverse. It is expected that the nature of this basic mechanism will be revealed in further studies of the bacterial systems as it is possible to examine this with a greater degree of precision, both at the chemical and the genetic level, than is usually

possible in many of the higher organisms.

It is the purpose of this report to consider a type of control of gene action by a system in maize ~~whose mode of action~~ that resembles that of some reported ~~cases~~ systems in bacteria. An "operator" controlling element is present at the locus of the gene $\underline{A}_2$ (associated with production of anthocyanin pigment in plant and kernel) in the short arm of chromosome 5. The independently located element of this system, comparable to the "~~repressor~~ regulator" element, discussed above, has been designated Suppressor-mutator and symbolozed as Spm. This system was first discovered when the operator was present at a gene locus associated with development of chlorophyll and locus was then designated $\underline{lu}^m$ for mutable luteus. Transposition of the operator element to the locus of $\underline{A}_2$ occurred in a plant having $\underline{lu}^m$. Following its insertion, there, the locus was designated $\underline{a}_2^{m-1}$, as it was the first case of mutability arising at the $\underline{A}_2$ locus in the Cold Spring Harbor cultures. Subsequently, the operator element appeard at the locus of $\underline{A}_1$ in the long arm of chromosome 3 in a kernel on the ear of a plant in the $\underline{a}_2^{m-1}$ culture and the modified locus was designated $\underline{a}_1^{m-1}$. Recently, the operator was inserted at the Wx locus in the short arm of chromosome 9 and it first appeared in a kernel on the ear of a plant carrying $\underline{a}_2^{m-1}$. This case received the symbol $\underline{wx}^{m-8}$ as it is the eighth case of instability

arising at the Wx locus ~~that has arisen and been examined~~ in the Cold Spring Harbor cultures. It has been possible, then, to examine the ~~mode of~~ operation of the Spm system at four different gene loci. It has been determined that the operator element, adjacent to the structural gene, controls the type of action of the gene that will be expressed in the presence of Spm and also the type that will be expressed in its absence. Basically, the mode of operation of this system is the same at all four gene loci (lu^m, a_2^{m-1}, a_1^{m-1} and wx^{m-8}). The original isolate, in each case, exhibited some degree of gene action in the absence of Spm and this action remained the same in successive cell and plant generations as long as Spm was absent. In the presence of Spm, however, gene action was suppressed until a mutation-inducing event occurred. With each of the original isolates, this event occurred in some cells, early in plant or kernel development, and there were two main consequences of it: mutation to a ~~stable~~ allele of the gene concerned, or a modification, probably affecting the operator element at the locus, that is reflected in subsequent cell and plant generations by altered responses of the locus both in the presence and in the absence of Spm. In the past, the latter modification has been termed "change in state" of the locus. In the presence of Spm, the altered states are distinguished, one from the

other, by difference in the time of occurrence of mutation-inducing events during development of a tissue, by the frequency of their occurrence at any one time, and by differences in types of stable alleles that result from the mutation-inducing events. They are also distinguishe one from the other, by the type of gene action that occurs in the absence of Spm, and among the many different states of a_1^{m-1} and a_2^{m-1} that have been isolated, a wide range is exhibited in type and intensity of anthocyanin pigment in plant and kernel in the absence of Spm, from no pigment with one state to near normal or to apparently normal A_2 type pigment production with others. Illustrations of this are given in figure 1. All those states that respond to Spm by producing stable mutations are grouped under the heading of the class I states. In addition, a state, designated class II, has arisen on several independent occasions from the original state of a_2^{m-1}. This state has been of considerable significance in the study of controlling elements in that its behavior mimics that of some of the described gene control systems in bacteria. In the absence of Spm (or when it is present but in its inactive phase, see below) this state of a_2^{m-1} produces anthocyanin pigment in plant and kernel that approaches in type and intensity that produced by the A_2 locus before the operator element entered it. When Spm is present and fully active, no anthocyanin is produced either in plant or kernel.

However, in contrast to the class I states, no gene mutations occur and no evidence has been found of removal of the operator from the locus by transposition, as occurs with the class I states. This statement is based on an analysis of the class II state in hundreds of plants and through 7 successive plant generations. Thus, this state appears to be quite stable. The action of the A_2 gene is "turned on" in the absence of Spm and "turned off" when it is and fully active present. It cannot be aruged successfully that the "turning off" of gene action, that is, the absence of pigment in plant and kernel when Spm is present and active, occurs at the level of the gene and under the direct control of the operator. Nevertheless it is clear, that the operator is necessary for the observed effect as no "turning off" of gene action occurs in the presence of Spm when the wild-type A_2 locus is present. The normal A_2 locus is present, and also an active Spm element the plants and kernels are fully pigmented.

The behavior of the Spm element likewise has been examined in detail. It undergoes mutation, transposition, and cyclically occurring change in phase of activity, that is, active to inactive and return to active. Some of the mutations of Spm result in a weakening of its capacity to induce mutation with the class I states without inducing a corresponding weakening of its suppressive action. Others result in a weakining of

Others result in a weakening both of its capacity to suppress gene action and its capacity to induce mutation with the class I states. Some of the mutants are highly stable whereas others are quite unstable, a return to full Spm expression occurring in some somatic cells during development in ~~different parts of a~~ both plant and kernel. Illustrations of these mutant types are ~~shown~~ given in figure 2. Several different methods have been used to examine transposition of Spm and these are reviewed elsewhere (McClintock, 1956). It has been learned that different isolates of Spm undergo transposition at different times in plant development. One isolate, extensively examined through three successive plant generations, undergoes transposition early in plant development. Other isolates, on the other hand, may rarely undergo transposition, either during ~~development of gamete~~ sporophytic or gametophytic development. Still other isolates may undergo transposition only late in plant development. It has been learned that a change from a fully active Spm to one that is weakly active, or the reverse, arises as the consequence of a single (mutational) event affecting Spm itself. It is not yet known, however, whether a mutational event is responsible for the expressed differences in time of occurrence of transposition of Spm during plant development and the frequency of this at any one time.

The third mentioned modification of Spm relates to its alternating cycles of activity within a plant and a description of this will be the main subject of this report. For example, a fully active Spm may be introduced into a zygote. As the plant develops from this zygote, the activty of Spm may be "turned off" completely in some cells. No evidence will be given of its presence in the descendent cells until, in some of them, Spm activity is "turned on" again. The presence of Spm will then be made evident in the descendents of these latter cells. The duration of any one phase of activity of Spm, -- either active or inactive -- may be long in some cases, or short in others. In some cases, the duration of one phase may extend over a number of plant generations whereas in others a succession of rather frequenty alterations in phase of activity may occur during the development of an individual plant. When a class I state of either a_1^{m-1} or a_2^{m-1} is present, and also an Spm with a long duration of its active phase, a ~~very regular~~ particular pattern of anthocyanin streaks in a non-pigmented background appears in the plant, and these streaks arise from mutation. The particular pattern, ~~in any one case, is a reflection of~~ produced effects of either a_1^{m-1} or a_2^{m-1} state that is present in the plant. If, however, the Spm element is undergoing frequent change in its phase of activity during the development of the plant, the pattern of anthocyanin distribution in the leaves, stalk, leaf and tassel of the mature plant may be exceedingly irregular. Pigment will appear in those areas

resulting from mutation-induced events at either a_1^{m-1} or a_2^{m-1}, whereas

of the plant in which Spm is in its inactive phase. Pigmented streaks in a non-pigmented background will appear in those areas of the plant in which Spm is in its active phase. However, the patterns of these pigmented streaks ~~in a non-pigmented background in these latter areas~~ may be quite different within different areas of the same plant. This is because no mutational events will occur at either a_1^{m-1} or a_2^{m-1} until Spm enters its active phase. If it enters this phase early in plant development, large pigmented areas may appear in a non-pigmented background with some of the class I states. If, however, it enters the active phase rather late in development, only small pigmented streaks will appear in a non-pigmented background. ~~If, however,~~ If now, the class II state of a_2^{m-1} is present in a plant, the distribution of pigmented and non-pigmented areas reflect for each area, regardless of its size, the particular phase of activity of Spm that is present in the cells of the area. In the non-pigmented areas, Spm is in its active phase and in the pigmented areas, Spm is in its inactive phase. Often, within a large nonpigmented area, a number of similiar sized, uniformly distributed pigmented streaks appear, Again, within a large pigmented area, a number of similar sized, uniformly distributed, ~~non~~pigmented streaks may appear. Such patterns of pigment distribution reflect the time and frequency of ~~occurrence~~ this any any one time, ~~of a change in phase of activity of Spm,~~

each arising as the consequence of a turning off of Spm activity

non-pigmented streaks often within the area

Thus, the occurrence of change in phase of activity of Spm

from active to inactive in the former example and from inactive to active in the latter example. Thus, the class II state of a_2^{m-1} has been particularly useful in examining cyclically occurring changes in phase of activity of Spm.

The Spm isolate, whose cyclically occurring changes in phase of activity have been examined in considerable detail, was the one that was present in a plant of the original culture having the class II state of a_2^{m-1}. It was followed through 7 successive generations but its behavior was examined intensively only during the last 4 of these generations. Originally, it was located in the short arm of chromosome 9 and was closely linked with the gene marker Wx. Very few stable cases of early occurring transposition of this Spm isolate have yet been detected, in tests of different parts of a large number of plants. However, it does transpose, and the time of occurrence of this appears to be confined mainly to late stages in sporophytic and gametophyt development. The frequency of occurrence of transposition is not very high, as will be indicated more evident later.

Before considering the experimental results that have made it possible to detect the alternating changes in phase of activity cycles of Spm, some mention should be made of the earlier studies of a_2^{m-1} and a_1^{m-1} and of the reasons for the differences encountered in ease of detection of the control system associated with each. Study of a_2^{m-1}

was undertaken before that of a_1^{m-1}. ~~However~~, an unequivocal ~~no clear evidence~~ was ~~obtained~~ of the mode of operation of the system ~~that was~~ responsible for control of gene action at a_2^{m-1}. was not recorded in the early studies. In contrast, progress in this respect was relatively rapid in the study of a_1^{m-1}. The reasons for this difference in ease of detection of the control system in the two cases ~~became clear~~ was made evident when it was realized that the Spm element in the original a_1^{m-1} culture had a very long duration of its active ~~xxxxxxxx~~ phase whereas the Spm element in the a_2^{m-1} cultures was undergoing frequent change in phase of activity during plant development. Confirmation of these differences in behavior of the Spm elements in the two cultures was obtained through interchange of this Spm elements between the a_2^{m-1} and a_1^{m-1} cultures, and also by isolation of Spm derivatives within each culture whose behavior was modified, either toward greater stability of a phase of activity or towards more frequently occurring changes in phase of activity.

EXPERIMENTAL ~~RESULTS~~ Procedures

Experiments aimed at elucidating the peculiar behavior of Spm commenced in the summer of 1956, utilizing for this purpose both the class I and class II states of a_2^{m-1}. This report will concentrate on those studies that utilized the class II state, and for reasons outlined above. In earlier studies of this ~~class II~~ state, it was learned that an apparent full A_2 gene expression would appear in plant and kernel in the absence of Spm, and that gene action would be suppressed in its presence. However, pigmented areas did appear in plants and kernels having this state of a_2^{m-1} and Spm. It was also realized that, in general, the pattern of such pigmented areas reflected the number of Spm elements that were present: the more Spm elements that were present, the fewer and smaller were the pigmented areas. Removal of Spm from some cells during development by the transposition mechanism might have been invoked to account for the appearance of the pigmented areas. However, the patterns of pigment distribution made it evident that removal of Spm ~~by transposition~~ could not be responsible for many of the pigmented areas that appeared. This was because within a fully pigmented area, smaller nonpigmented areas often were present and within some of these latter, in turn, pigmented streaks appeared. One set of tests that have made it possible to understand the way in which these patterns and phenomena

commenced in the summer of 1956 with progeny of plants in culture number 7109, to be described below.

The plants in culture number 7109 originated from variegated kernels (pigmented spots in a colorless background) on an ear of a plant that had the following constitution: a_2^{m-1} (class II st te) Bt/a_2 bt in chromosomes 5, Wx +/wx Spm in chromosomes 9, and one additional Spm not linked to m rkers in either of these chromosomes. The silks of this ear had received pollen from a plant that was homozygous for a_2, bt, and Wx and had no Spm. Among the a_2^{m-1} carrying class of kernels on the ear, there were fully pigmented kernels and variegated kernels, that is, those that had spots of anthocyanin pigment in a colorless background. Some of the variegated kernels showed only specks of pigment and were thought to have received both of the Spm elements that were present in the female parent, and other had a number of larger spots of pigment and the kernels showing this pattern were thought to have received only one of the two Spm element that were present in the female parent. Plants were grown from the two classes of variegated kernels, 5 from kernels of the first type and 6 from kernels of the latter type. A number of different types of test crosses were conducted with each plant. Confirmation was obtained from these tests of the presence of two Spm elements in the plants derived from the former mentioned type of

variegated kernel and of the presence of one Spm in each of the six plants derived from the latter type of variegated kernel. This report will consider mainly the tests that were conducted in successive years with ~~prog~~ progeny stemming from three of these latter six plants, that is, those that arose from kernels that showed many pigmented spots in a colorless background. The constitution of these three plants proved to be as follows: two plants were a_2^{m-1} Bt/a_2 bt, Wx +/wx Spm (plants 7109B-1 and B-2) and one plant (7109C-4) was a_2^{m-1} Bt/a_2 bt, Wx/wx, and had 1 Spm, not carried in the short arm of chromosome 9.

The appearance of the kernels on ears produced by the six plant in culture 7109 B and C (that is, those that ~~were considered to have~~ had one Spm ~~in each~~) when crossed reciprocally with plants homozygous for a_2, bt, and Wx and having no Spm, are entered in table 1. A clearly expressed 1 : 1 ratio of fully pigmented kernels to kernels that showed pigmented spots in a colorless background appeared on the ears produced by all tillers of these plants except that of tiller-1 of plant C-1. However, a pronounced deviation in favor of the pigmented class appeared among the kernels on the ear produced by the main stalk of plants 7109B-1 and 7109C-3, and to a lesser extent on this ear produced by plants 7109C-1

and C-2. Many of the kernels in the pigmented class were uniformly and deeply pigmented but in some kernels placed in this class in the table, the intensity of pigment over the aleurone layer was not uniform. Lightly pigmented areas or even some colorless areas were present but sharply defined borders between areas with different pigment intensities, or between pigmented and nonpigmented areas were not usual. This gave the kernels a diffusly-mottled appearance (see photo, figure). At the time of observation of these kernels, no attempt was made to place in a separate class those kernels exhibiting different grades of this diffusely-mottled phenotype as it was evident that this class graded into the fully and uniformly pigmented class. Subsequent tests proved, however, that all of the plants derived from the diffusley-mottled kernels carried an Spm element in them as did some of the plants derived from kernels on these ears that were uniformly and deeply pigmented.

The ear of tiller-1 of plant 7109B-2 was self-pollinated. Among the 367 Bt kernels on the resulting ear only 23 were completely colorless. Sixteen of these colorless kernels were Wx and 7 were wx. Among the remaining 344 Bt kernels, 87 were ~~uniformly~~ deeply and uniformly pigmented of which 84 were Wx and 3 were wx. One Bt kernel exhibited the diffuse-mottled phenotype, described above, and it was Wx. All of the remaining 256 Bt kernels were variegated in that pigmented spots appeared in a colorless background. These kernels ~~were~~ could be divided, roughly, into three classes: those that showed only a few specks of pigment, of which 11 were Wx and 20 were wx, those that had a number of larger spots as well as some specks of pigment, of which 105 were Wx and 42 were wx, and those that exhibited some quite large pigmented areas as well as a number of smaller pigmented ~~areas~~ spots, of which64 were Wx and 14 were wx. Other crosses conducted with plants 7109B-1 and 7109B-2 that need not be outlined here, had indicated that in both ~~plants~~ of them one Spm was present and that it was located close to wx in chromosome 9. On the self-pollinated ear of the tiller of plant 7109B-2 the ratio of 84 Wx : 3 wx among the uniformly pigmented kernels and of 180 Wx to 76 wx among the variegated class was in conformity with this placement of Spm.

Plants were grown in the summer of 1957, under culture number 7308, from some of the kernels in the uniformly

pigmented Bt, Wx class, from some of the variegated kernels in the Bt, Wx and in the Bt, wx classes and from 5 kernels in the colorless, bt, wx class. Plants derived from the latter class of kernels were expected to be homozygous for a_2, bt, and wx, and to carry Spm at a known location in chromosome 9. Only two of the five plants survived. In both of them, a single Spm element was present, and subsequent tests indicated that it was located close to wx in one chromosome 9. These two plants, 7308D-1 and D-2, were extensively used as pollen parents in crosses to plants carrying not only the class II state of a_2^{m-1} but also to plants carrying a class I state of a_2^{m-1}. The behavior in subsequent generations of the Spm element in each of these two plants (see C and D of table 3) has contributed much to an understanding of cyclical changes in phase of activity of Spm, as will be made evident later.

Because hundreded of plants have been tested to determine whether or not Spm was present in them, and if so, the phase of its activity in different parts of the same plant, it will not be feasible to give a detailed account of the results obtained from each such test. Therefore, examples will be chosen that will illustrate the nature of the tests that were conducted with these plants and the conclusions that may be drawn from each. Before doing so, the reader is advised to inspect table 2

which summarizes the results obtained from tests that were conducted through successive generations with the Spm element that was present in plant 7109B-1. This plant had one Spm element closely linked with wx in the short arm of chromosome 9. The Spm element was in its inactive phase in the cells that gave rise to the ear of the main stalk. This was indicated by the uniform distribution of anthocyanin pigmentation in the main stalk of this plant and also by the phenotypes of the kernels on the ear it produced (table 1). However, the phenotypes of a few of the kernels on this ear indicated that Spm was present in the cells that contributed to this ear and that it had entered its active phase very early in development of a few of them, or had entered the active phase later in development in some cells of other kernels. In the cells that gave rise to the ear on each of the three tillers of plant 7109B-1, Spm was in its active phase. This was made evident not only by the appearance of these tillers, in that they exhibited streaks of anthocyanin pigment in a nonpigmented background, but also by the ratio of kernel types on the ears produced by each (table 1). The pollen of the main stalk of plant 7109B-1 was also used in making crosses and it was made evident from the types of kernels on the resulting ears that some grains carried Spm in its active phase whereas some others carried Spm in its inactive phase.

Table 2 included the results of tests only of those progeny of plant 7109B-1 that carried Spm. A number of progeny plants that did not carry Spm was also tested and the methods used to determine whether Spm is absent in a plant or is present in its inactive phase will be considered shortly. In this table, the symbol "-" indicates that Spm was inactive not only in the cells that gave rise to the ear, but also in th cells that gave rise to the aleurone layer in all kernels on the ear. The symbol "vd" (very delayed return to the active phase) indicates that Spm was inactive in the cells that gave rise to the ear and also in those that gave rise to nearly all of the kernels on the ear; a return to the active phase was seen only in some parts of a few kernels on these ears. The symbol "d" (delayed return to the active phase) indicates that the Spm element was inactive in the cells that gave rise to the ear, but that it had changed to the active phase in a number of kernels on the ear, being in its active phase in some of them at the start of endosperm development. The symbol "+" indicates that Spm was in its active phase in the cells that gave rise to the ear, turning to its inactive phase in some cells during endosperm development. The symbol (+ -) indicates that a part of the ear arose from cells in which Spm was in its active phase whereas another part arose from cells in which Spm was in its inactive phase. Ears of this type exhibited sharply defined sectors

with Spm active in one sector and inactive in the other.

Table 2 reveals that the inactive phase of Spm, present in the main stalk of plant 7109B-1, remained in this phase in the tested parts of plants over four successive plant generations, and exhibited the same pattern of behavior in each generation. In plants having this inactive Spm, return of it to the active phase was very much delayed. In general, visual evidence of this in the plant was given only by tillers. In some tillers, the change of Spm from inactive to active was exhibited by the presence of nonpigmented sectors in an otherwise pigmented tiller, and within some of these latter sectors, in turn, small pigmented streaks were present.

In this study of alternating changes in phase of activity of Spm, a number of tests were conducted to determine whether or not the presence of an active Spm in the same nucleus with an inactive Spm would effect a change in the latter. Evidence obtained from tests of this type indicate that the active Spm does not initiate change in phase of the inactive Spm nor does it appear to alter the duration of an inactive phase. One such test, conducted with the inactive Spm originally present in the main stalk of plant 7109B-1, is recorded in A of table 2 under culture number 7780A, Year 1960. The plants in this culture arose from a_2^{m-1} carrying kernels

on an ear of plant 7599B-4 (see Year 1958, Table 2 A) that was a_2^{m-1} (class II) Bt/ a_2 bt, Wx +/wx Spm-inactive in constitution when crossed by a plant that was homozygous for a_2, bt, and wx, and that had an active Spm linked with wx in one chromosome 9. The plants in culture 7780A grew from kernels selected from this ear because each had received the inactive Spm from the female parent (originally derived from the main stalk of plant 7109B-1) and the active Spm element from the male parent (this Spm originally derived from tiller-1 of plant 7109B-2). The plant in B of culture 7780 were derived from kernels that had received the active Spm from the male parent but no Spm from the female parent. As the table indicates, the inactive Spm, originally present in the main stalk of plant 7109B-1, appeared in the progney plants of culture 7780A and exhibited quite the same behavior with respect to phase as it exhibited in the ancestor plants in which it was the only Spm element that was present.

The same Spm element that was in an inactive phase in the main stalk of plant 7109B-1 was in an active phase in the cells that gave rise to the ear of each of the tillers of this plant (table 1). The activity phases of this Spm in cells giving rise to ears in successive generations of plants are recorded in B of table 2. In the table, the letter T (transposition) before the plant number indicates that the plant carried this

Spm at a new location in the chromosome complement. The plants in B of culture 7306 (Year 1957) were derived from the very few kernels on the ear of tiller-1 that were suspected to have received more than one Spm element from the 7109B-1 parent plant, the additional Spm having arisen through the transposition mechanism. The plants in culture 7560 (Year 1958) were derived from the recombinant class of kernels on the second ear of the main stalk of plant 7306A-1. As indicated in a previous publication (McClintock, 1956) the phenotypic recombinant class may be a composite of individuals some of which represent the true crossover class and other of which carry a transposed Spm element, and do not represent the true crossover class. It will be noted that the behavior of Spm derived from tiller-1 of plant 7109B-1, whether in its original location or transposed, with regard to phase of activity in the cells that gave rise to the tested ears in B of table 2, is quite different from that of the same Spm element in an inactive phase, derived from the main stalk of plant 7109B-1 and registered in A of this table. Change from the active to the inactive phase occurred in some cells early in plant development, but in many other parts of the plant, such change was delayed, occurring in some cells only late in plant development.

Similar types of test as those outlined above, were conducted with the Spm carrying progeny of tiller-2 of plant 7109B-1. Spm was in its active phase in the cells that gave rise to the ear of this tiller. Its behavior in successive generations is recorded in C of table 2. In general, its behavior was similar to that in tiller-1. Only two comments regarding this need be made here. Firstly, it may be pointed out that tests of Spm location in eight plants derived from the recombinant class, entered in cultures 7561, and 7562, Year 1958, did not reveal a case of transposition among them. The second comment is directed to the first ear of the main stalk of plant 7561-4. The constitution of this plant was a_2^{m-1} (class II) Bt/a_2 bt, Wx Spm/wx +. The silks of the first ear of the main stalk of this plant received pollen from a plant homozygous for a_2, bt, and wx, and in which no Spm was present. The resulting ear was sectorial. Spm was in its active phase in all parts of this ear except for a sector in the middle of the ear in which no evidence of Spm was given by any of the a_2^{m-1} carrying kernels within it. Kernels with a_2^{m-1} that were also Bt and Wx and having an active Spm in them, derived from that part of the ear in which Spm was active, were sown in 1960 under culture number 7777-A and B. Fully pigmented (a_2^{m-1}) carrying kernels that were Bt and Wx were sown under

C and D of culture 7777. All of the plants in culture 7777 carried Spm in the Wx chromosome contributed by the female parent. However, it was totally inactive in all of the cells that contributed to each of the tested ears of plants in C and D of culture 7777 and remaind in the inactive phase during development of all but a very few kernels on these ears. In the leaves of these plants, several small sectors were present in which Spm had changed from its inactive to its active phase in the cell that gave rise to each such sector. In most of the plants, these small sectors appeared only in tillers or in parts of the support roots of the main stalk. It was obvious, however, that the duration of this particular inactive phase was long. In contrast to this the same Spm element in the plants of A and B of culture 7777 was undergoing frequent change in phase of its activity.

As mentioned earlier, the pollen taken from the tassel produced by the main stalk of plant 7109B-1 contained some grains in which Spm was in its inactive phase and others in which it was in its active phase. It is presumed that the tassel was sectorial with regard to phase of activity of Spm. Tests of the phase of activity of Spm in progeny produced by use of this pollen is given in D of table 2.

In order to obtain evidence of Spm activity in cells that gave rise to the ears summarized in table 2, it was necessary in making the cross to each ear to use pollen from a plant having a particular combination of markers. With regard to such marker, the tested plants were of two main types: those that were homozygous for a_2 and bt (and also for some other markers that need not be considered at this time), and those that carried in their chromosome 5 a class I state of a_2^{m-1} (three different class I states selected for this purpose), or a class II state of a_2^{m-1}, and also bt. These a_2^{m-1} carrying tester plants were homozygous for wx and they had no Spm. The tester plants, homozygous for a_2 and bt, wereof three main types: homozygous for wx and having no Spm (type-1), homozygous for wx and having no Spm (type-2), and homozygous for wx and carrying one or more Spm elements each in its active phase (type-3). Most of the latter plants carried one Spm, linked with wx in one chromosome 9. Some had two Spm elements located close to wx in each chromosome 9 (designated Spm/Spm in the tables) or two Spm, one located close to wx in one chromosome 9 and the other located elsewhere in the chromosome complement (designated Spm + Spm in the tables), the latter having been transposed from a location close to wx to a new location. Each type of tester plant served a purpose in examining Spm behavior.